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EARLY MORPHOLOGICAL CHANGES PRODUCED BY ANTI-INFLAMMATORY STEROIDS ON TISSUE CULTURE FIBROBLASTS*

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The fibroblast, a widely distributed cell of mesenchymal origin, is the most abundant cell type of loose connective tissue (1). Although these cells participate in many functions, their name refers to their ability to elaborate connective tissue fibers. The fibroblast's major activity is exhibited in the formation of new connective tissue components during growth, regeneration and repair (2). These cells have been shown, by Dougherty *et al.*, to play a major part during the development of inflammatory reactions (3–5). Fibroblasts are extremely sensitive target cells to anti-inflammatory steroids (6–8). The growth inhibitory effects of anti-inflammatory steroids on conective tissue cells *in vitro* (6–10) and *in vivo* (11, 12) is a well-known phenomenon. The following is a description of the early morphological changes occurring in tissue culture fibroblasts, in the presence of the most potent naturally occurring steroid, cortisol, and the most potent topical synthetic anti-inflammatory steroid, fluocinolone acetonide (13–15).

METHODS

Fibroblast L-929 were grown in Eagle's media supplemented with 10% calf serum, 100 i.u. of penicillin, and 100 μg of streptomycin sulfate per ml. The steroids used were dissolved in 2 μl of propylene glycol to give final concentrations of 1.2 μg of fluocinolone acetonide; 5.0 μg of cortisol; 10 μg of tetrahydrocortisol and 10 μg of 11-deoxycortisol per ml of growth media. Figure 1 represents the chemical structures of steroids used in the present study.

Twenty-five thousand fibroblasts were inoculated into Leighton tubes containing cover slips (10 x 35 mm) and allowed to grow for 48 hours. Then each cover slip was removed from the tube and rinsed with warm media (media + vehicle for the controls and media + steroid solution for the experimentals), mounted on a standard microscopic slide, following with the addition of a small amount of the corresponding media. The cover slip was sealed with petrolatum and immediately placed in a temperature controlled microscope at 38° C. Photographic procedures were initiated immediately after the locating of suitable cells. Phase contrast photomicrographs were taken at 15 minute intervals during four hours.

The steroid solution was in contact with the cells from the time of their removal from the Leighton tubes until the end of the period of observation.

RESULTS

To conserve space, we will not present the complete sequence of photomicrographs. Pictures 1 to 6 represent the sequence of events occurring in fibroblasts in the presence of normal media. Picture 1 shows the appearance of the culture at the beginning of the observation. Picture 2 was taken 30 minutes later. Pictures 3, 4, 5 and 6 were taken at one hour intervals. During this time, all cells presented the normal characteristic shape and motility of L-929 fibroblasts. This control study demonstrates the viability of the cells during the entire period of observation.

Pictures 7 through 12 show the progressive changes occurring at the same time interval as shown in the controls (30 minutes; 1, 2, 3, and 4 hours) in the presence of 5.0 μg of cortisol per ml of growth media. Notice the regression of cytoplasmic prolongation during the latter part of the experiment.

Pictures 13 to 18 show the cellular changes in the presence of 1.2 μg of fluocinolone acetonide. The time intervals are the same as in the control series. At 30 minutes there is little change as compared to time zero. After one hour (Picture 15) the smaller fibroblasts start to show a definite change in shape; however, the larger ones still remain more or less unchanged. In Pictures 17 and 18 (3 and 4 hours, respectively) the size and shape of all the fibroblasts in the preparation have shown considerable change. The reduction in size, greater refrac-

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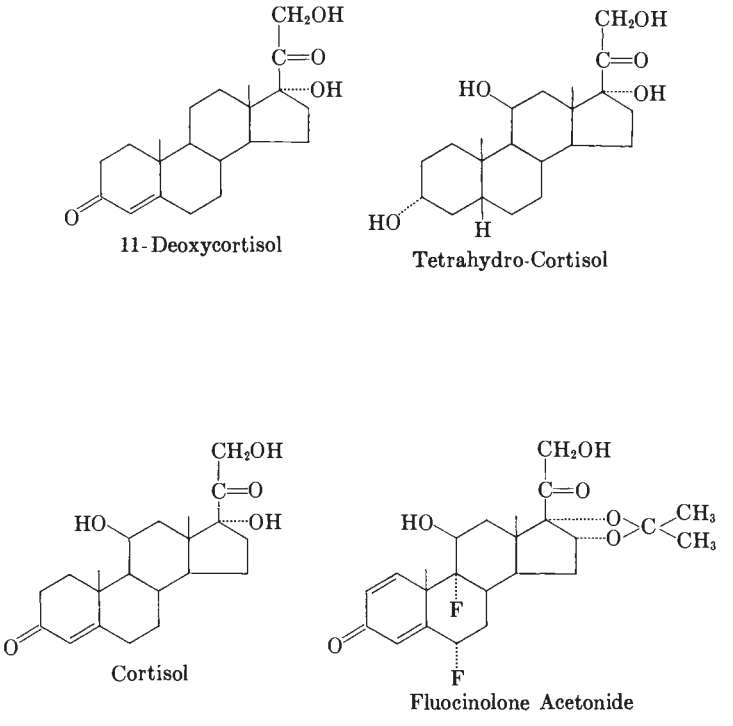


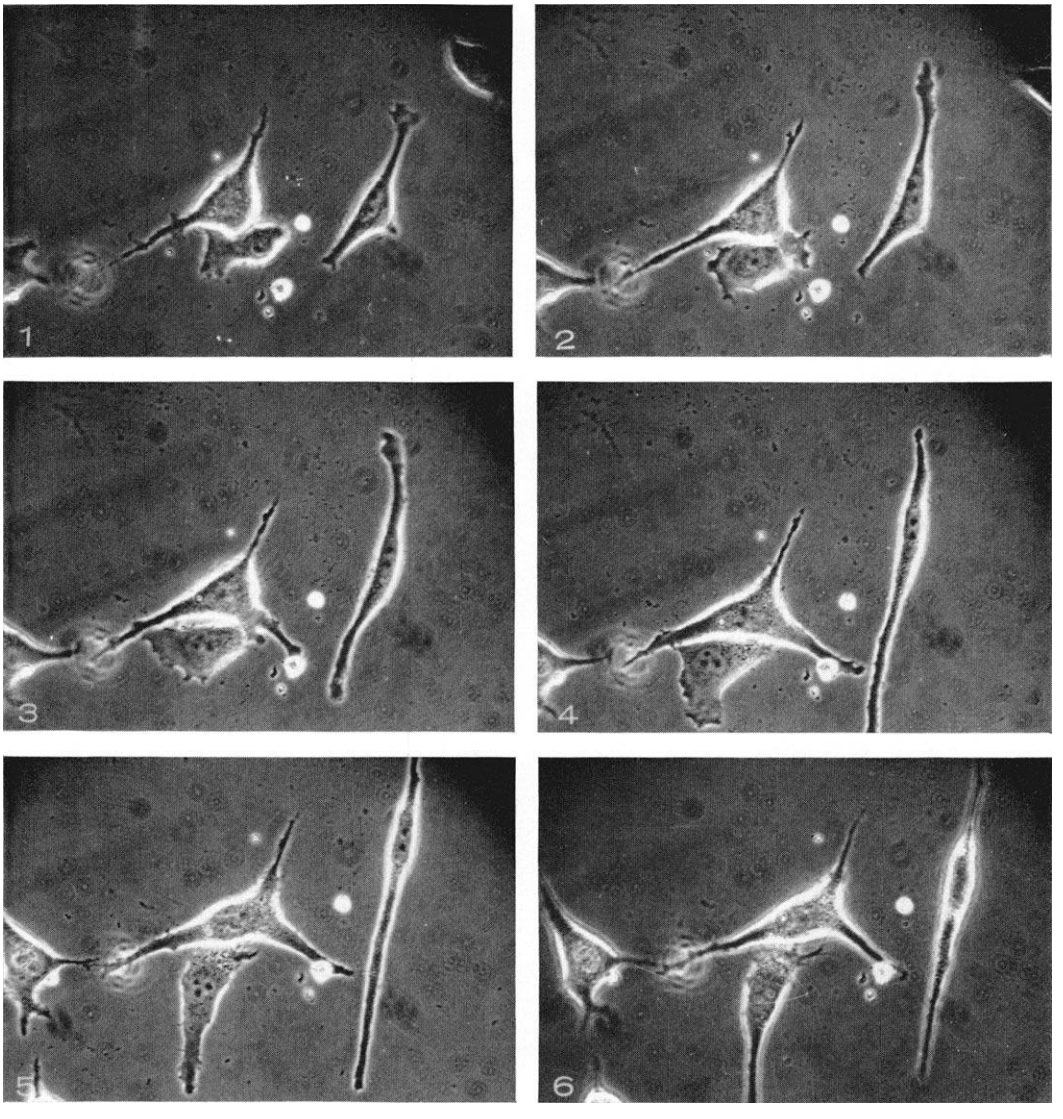
FIG. 1

tility, and some globular forms of the small and medium size fibroblasts is clearly shown. Noteworthy is the shortening of cytoplasmic prolongations. Fibroblasts treated with tetrahydrocortisol and 11-deoxycortisol do not show any change in morphology, but behave in a manner similar to the controls.

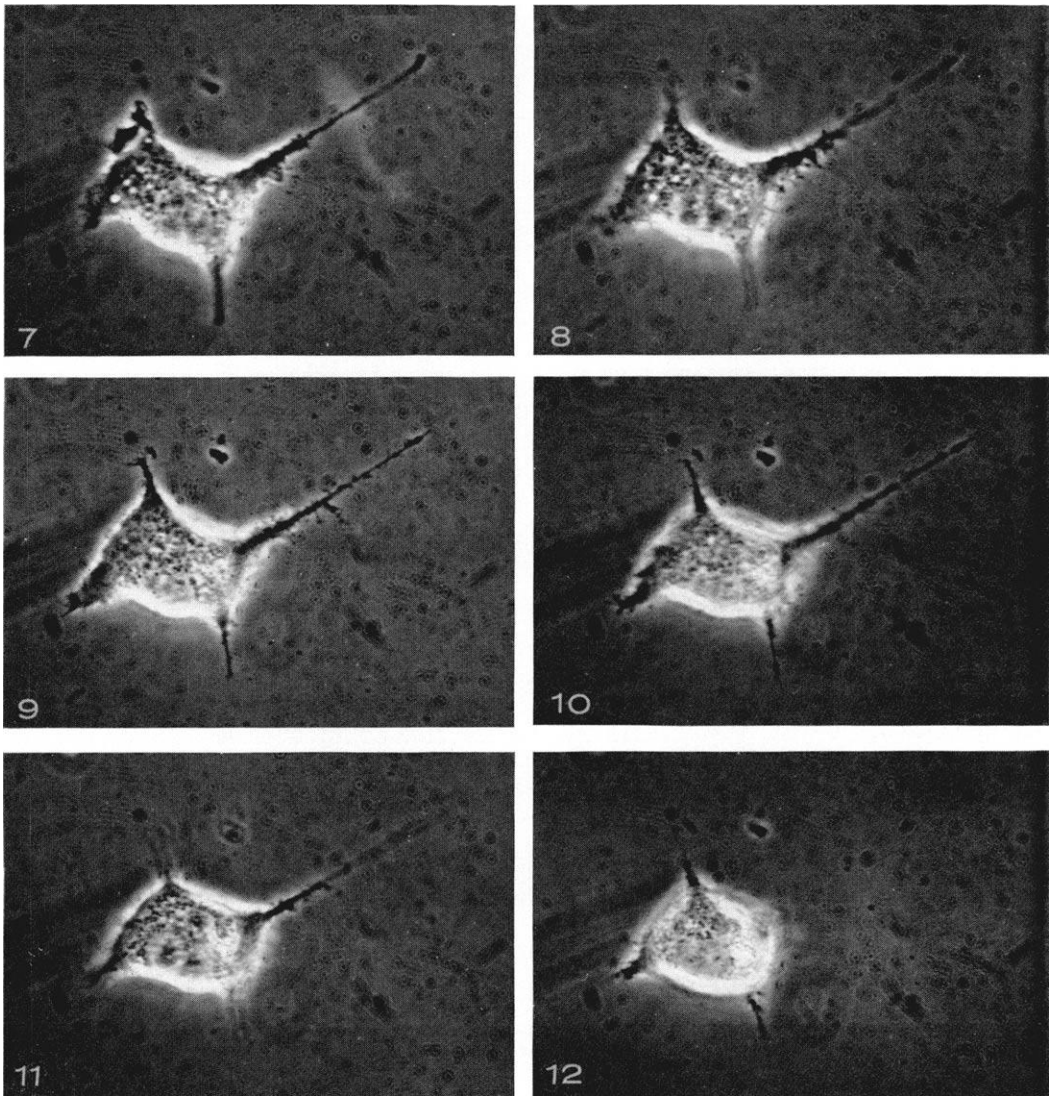
DISCUSSION

The fibroblast has been shown to be a very sensitive target cell for corticosteroids (6–8, 12). It has been demonstrated previously that formation of collagen and mucopolysaccharides by fibroblasts is inhibited in the presence of corticosteroids (17, 18). Also, the direct influence of anti-inflammatory steroids on fibroblasts seems to play a protective role during the development of inflammatory processes (3, 5). Steroid induced morphological fibroblastic changes in normal and inflammed tissue *in vivo* (3–5) are similar to the ones observed in this study *in vitro*. Holden and Adams (8) have previously described the shortening of terminal processes of tissue culture cells as well as the tendency of cellular aggregation, when treated with high concentration of anti-inflammatory steroids (10–50 μg of cortisol per ml). Ruhmann and Berliner (6) have shown the same phenomenon, observing the maximum effect five days after treatment with 0.1 μg of cortisol per ml. Utilizing fluocinolone acetonide at concentrations of 0.00075 μg per ml, duplication of these morphological changes can be obtained (6). A bioassay procedure has been developed by which the structure-activity relationship of various anti-inflammatory steroids can be correlated with their growth inhibitory effects upon tissue culture fibroblasts (19).

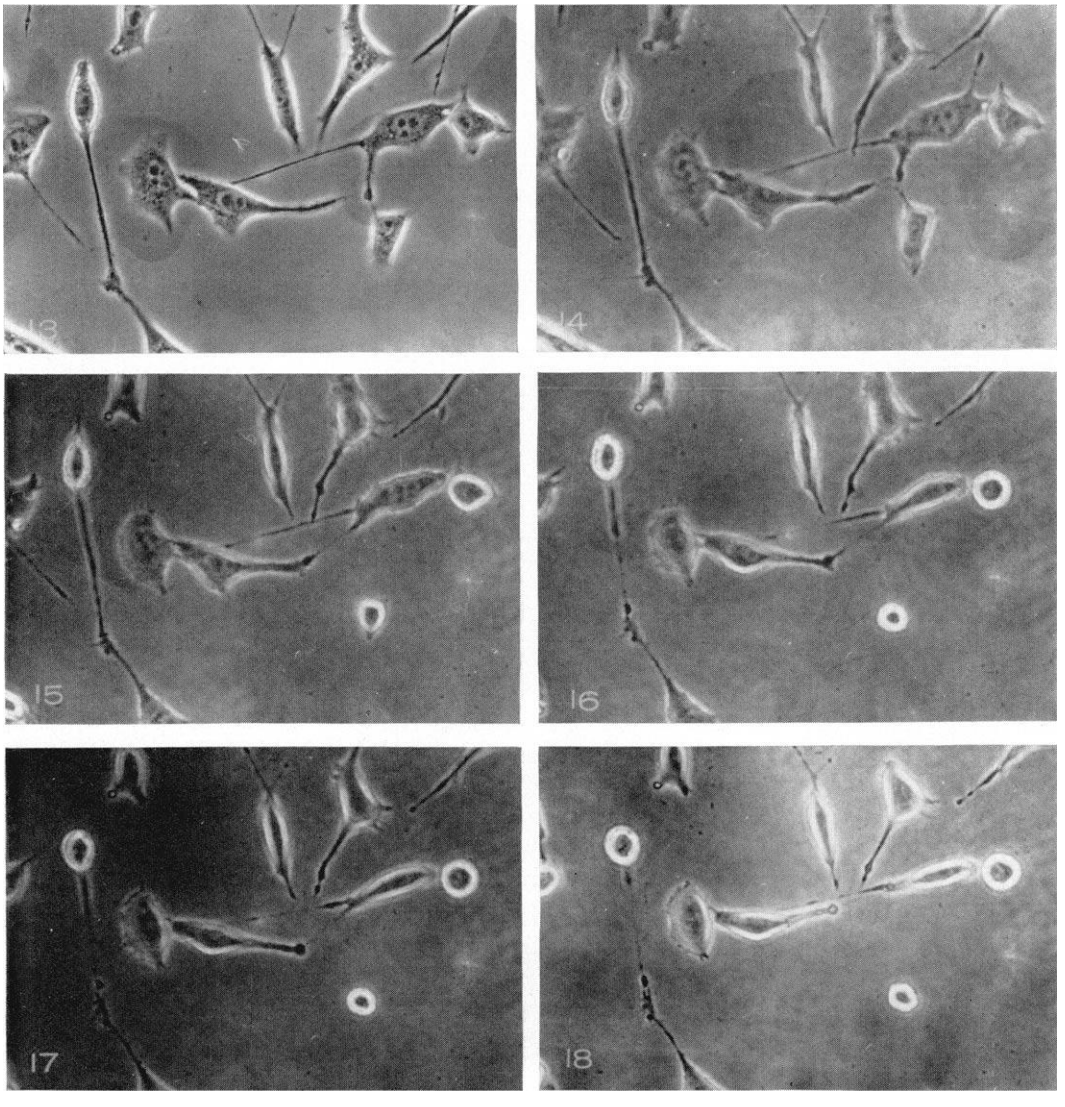
In the present study we are describing the earliest time at which we can obtain this phenomenon. After 3 hours of treatment with cortisol or 1 hour with fluocinolone acetonide, some morphological changes in tissue culture fibroblasts are apparent. Loss of the characteristic spindle-like shape, as a result of increased cellular refractivity and the assumption of smaller globular forms. Smaller amounts of fluocinolone acetonide than cortisol are needed to produce similar morphological fibroblastic alterations. Treatments of these cells with other steroids such as tetrahydrocortisol and 11-deoxycortisol does not induce similar morphological changes.



PICTURES 1 TO 6. Normal L-929 fibroblast. High power approximately × 369



PICTURES 7 TO 12. Cortisol treated L-929 fibroblast. High power approximately × 855



PICTURES 13 TO 18. Fluocinolone acetonide treated L-929 fibroblasts. High power approximately × 369.

Moreover, these compounds are not active anti-inflammatory agents. At this time, it is difficult to correlate the mechanism of the cells' rounding up with the salutary effectiveness of corticosteroids as anti-inflammatory agents. While other agents may cause fibroblasts to round up, inflammatory steroids like fluocinolone acetonide appear to initiate this process as an essential part of their antiphlogistic activity. In inflammation it has been postulated by Dougherty *et al.*, (3–5, 20) that the rounding up effect produced by anti-inflammatory steroids upon fibroblasts is a protective one. This morphologic form appears to enable the fibroblast to resist the chain reaction of cell destruction initiated by the inflammatory agent and the cytotoxic products elaborated during inflammation. An interesting correlation can be obtained, since the same structure-activity relationships exist in the *in vivo* studies on corticosteroid anti-inflammatory activity as in the *in vitro* studies presented in the present investigation.

SUMMARY

Fibroblasts L-929 grown in tissue culture, exhibited the typical morphological characteristics when treated with active anti-inflammatory steroids. The same phenomenon has been described to occur *in vivo*. These morphological changes consist in the formation of round fibroblasts. Fluocinolone acetonide produces this effect at lower concentration and at an earlier time than cortisol.

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